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# Antibiotic Action of Trypsin Inhibitors.

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In a previous communication[1] evidence was presented which demonstrated that the *in vitro* action of hemolytic streptococcal fibrinolysins was inhibited in the presence of protease inhibitors ("Trypsin-inhibitor" of Northrop and Kunitz[2] and soybean "antitrypsin"[3]). Irrespective of whether streptococcal fibrinolysin is an enzyme or, as has been suggested recently, an "activator" of human serum protease (tryptase), it is a definite metabolic product of streptococci which is believed to play some role in virulence of infection. Consequently, it became of interest to investigate *in vitro* the action of protease inhibitors on hemolytic streptococci and other bacteria.

0.1 ml of suitable dilutions of an 18-hour culture of the microörgnisms was transferred to 0.9 ml saline in which was dissolved from 10 to 50 mg of crystalline "trypsin-inhibitor" compound, making a total concentration of from 1 to 5% of the inhibitor in the solution. Dr. M. Kunitz provided some of the crystalline compound used in this study. Since the "trypsin-inhibitor" compound contains at least 50% $MgSO_4$, controls were set up with a similar concentration of MgSO. The tubes were incubated at 37°C and at various intervals 0.1 ml was transferred to 1 ml broth and to 10 ml blood agar, a pour plate being made from the latter. Twenty-four hours later, the broth and plates were examined. Various dilutions of the broth culture were then transferred to a constant amount of blood agar and a pour plate prepared, a colony count being made 24 hours later.

It was observed that in concentrations of from 1 to 5% of crystalline trypsin inhibitor

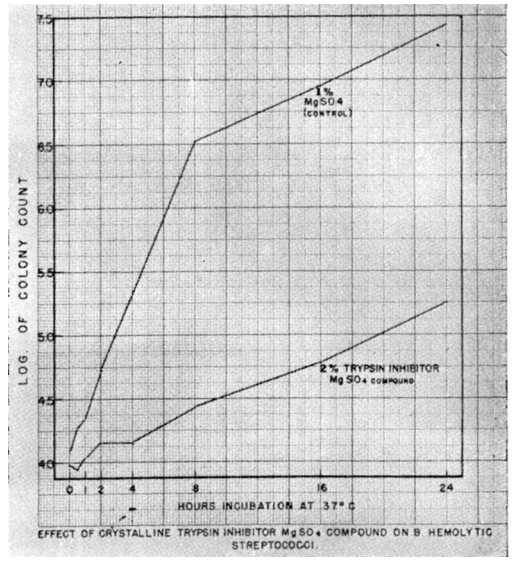


Fig. 1.

$MgSO_4$ compound, growth of hemolytic streptococci did not occur or was markedly depressed after incubation of from 4 to 8 hours. Fig. 1 depicts a growth curve of such an experiment with streptococci, the curve being based on colony counts made on pour plates of suitable dilutions prepared at stated intervals and incubated 18-24 hours at 37°C. Table I depicts another typical experiment.

Studies with soybean "antitrypsin" preparations were uniformly negative, presumably due to the lack of purity for in most instances an actual stimulation of growth could be demonstrated. Relatively crude preparations of pancreatic trypsin-inhibitor gave results similar to those of the crystalline inhibitor.

In view of the observations with streptococci other microörganisms were studied. *Staphylococcus aureus* and *Escherichia coli* were utilized for these further studies. The results were identical with those obtained with streptococci

[1] Mirsky, I. A., *Science*, 1944, **100**, 198.

[2] Northrop, J. H., and Kunitz, M., *J. Gen. Physiol.*, 1932-33, **6**, 267.

[3] Ham, W. D., and Sandstedt, R. M., *J. Biol. Chem.*, 1944, **154**, 505.

TABLE I.
Effect of Trypsin-Inhibitor on B Hemolytic Streptococci.

| Incubation period before plating, hr | Colony count* | |
|---|---|---|
| | Trypsin-inhibitor $MgSO_4$ compound 5% | $MgSO_4$ 5% |
| 2 | † | † |
| 4 | 656 | † |
| 6 | 23 | † |
| 8 | 0 | † |

* 0.1 cc of 1:1000 dilution of 18-hour culture removed at stated intervals after incubation with test substance and transferred to blood agar pour plates and incubated at 37°C for 24 hours.
† Too many to count.

in that inhibition of growth ensued on incubation with pancreatic trypsin-inhibitor.

In an excellent study of the effect of trypsin inhibitor on bacterial growth, Grob[4] demonstrated that growth was "somewhat retarded" in the presence of the inhibitor. He concluded that the trypsin-inhibitor had no bacteriostatic action but affected growth through the prevention of proteolysis in the medium. However, he could not demonstrate any action on *Staphylococcus aureus*. Our studies suggest that the inhibitor can be bacteriostatic when employed in adequate concentration (Table I).

It is obvious that the trypsin-inhibitor compound is not nearly as potent an antibiotic agent as is penicillin and other antibiotics. However, the relative ineffectiveness of penicillin on *E. coli* and the fact that trypsin-inhibitor affects *E. coli* to the same degree as hemolytic streptococci suggests a greater non-specificity of the antibiotic action of the protease inhibitor.

Unpublished data reveal that penicillin has a weak but definite antiproteolytic action as do Actinomycin A and Tyrothricin.[5] It is probable that the efficacy of penicillin as an antibiotic agent lies in its ability to inhibit enzymes other than the proteases. On the other hand, the effect of trypsin-inhibitor can be attributed solely to its inhibition of the bacterial proteases since it has been observed that this protease inhibitor is not specific but can inhibit a number of proteolytic enzymes. Further, the fact that synthetic detergents which are antiproteolytic are also germicidal lends support to this hypothesis.

The significance of the above observations and those of Grob lies not so much in the demonstration that the protease inhibitor has an antibiotic action as in the suggestion that the rise of the antiprotease titer of blood which occurs in a variety of pathologic states in man may play an important role in "resistance" to infection. In this respect the role of numerous other anti-enzymes known to be present *in vivo* merits intensive investigation.

*Summary.* Trypsin-inhibitor has antibiotic properties.

We wish to express our appreciation of the courtesies and facilities extended by Dr. T. Duckett Jones and the staff of the House of the Good Samaritan, and of our indebtedness to Col. W. Paul Holbrook for his interest and cooperation.

[4] Grob, D., *J. Gen. Physiol.*, 1943, **26**, 431.

[5] Neter, E., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 163.

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## Alloxan Administration to the Duck.

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One of the perplexing problems in carbohydrate metabolism is the fact that pancreatectomy does not result in the development of diabetes mellitus in the duck or chicken. Further, the administration of crude saline extracts of the anterior pituitary gland to such